complement and phagocytes and therefore useful for conferring passive immunity. One source is individuals immunized with any one of the immunogenic compositions described in the specification. Such passive immunity is disclosed, for example, in the specification at page 5, lines 32 to page 6, line 4.

> The immunogenic compositions of this invention are capable of eliciting active and passive protection against infection by group A streptococcal infection. For passive protection, immunogenic antibodies are produced by immunizing a mammal with a vaccine made of the immunogenic composition of the invention and then recovering the immunogenic antibodies from the mammal.

See also:

> The immunogenic antibodies used for passive protection are produced by immunizing a mammal with any of the immunogenic composition of the invention and then recovering the bactericidal antibodies in a gamma globulin fraction or as serum, or as specific antibodies from the mammals. As used herein, the vaccines of this invention are capable of eliciting antibodies useful or providing protection against infection of group A Streptococcal bacteria.

Page 17, line 33 to page 18, line 6.

Another source of antibodies disclosed in the application and discovered by the applicant to be bactericidal in the presence of complement and phagocytes are individuals who have not been vaccinated with any of the immunogenic compositions of this invention but yet have titers of antibodies having bactericidal results against group A Streptococcus.

For example, the specification discloses that antibodies to the group A carbohydrate antigen are readily detected in human sera and that the titer of these antibodies is age dependent. Page 10, lines 7-12. Furthermore, the specification discloses that these naturally occurring antibodies produce a bactericidal effect resulting from their opsonphagocytic activity. Page 10, lines 17-22. See also Figure 4 of Example 1 which discloses that the group

A carbohydrate antibodies present in human sera are phagocytic and therefore bactericidal against group A Streptococcal bacteria. Page 24, lines 1-28.

Claims 73 through 79 find support in applicants' disclosure which is the first teaching of the functional equivalence of antibodies raised against the group A Streptococcal polysaccharide epitope identified in the instant application and antibodies obtained from individuals with naturally acquired immunity. In addition, the specification discloses that both naturally occurring antibodies and those raised in response to the claimed vaccines are opsonophagocytic and lead to a bactericidal response in the presence of complement and phagocytes. According to the specification, antibodies from the sera of an individual may be isolated using N-acetylglucosamine coupled sepharose beads as follows:

> Absorption of N-acetylglucosamine antibodies from human sera: 600 μl of a 50% suspension of a N-acetylglucosamine coupled to Sepharose beads (Sigma Chemical Co.) in PBS was placed into a sterile eppendorf tube and centrifuged at 4°C at 14,000 RPM for 10 minutes. The supernatant was removed and 300 μl of serum added to the beads. The suspension was rotated end over end for 1 hour at 37°C. Following a second centrifugation under the same conditions, the absorbed serum was removed and used in the bactericidal assay as described previously. To remove the N-acetylglucosamine antibodies from the affinity column, the beads containing the absorbed antibodies were packed in a 1 ml tuberculin syringe over which a solution of 0.58% (v/v) glacial acetic acid in 0.15 M NaCl, pH 2.2 is passed. The eluant is monitored by absorption at 280 nm and the peak fractions collected, dialyzed against PBS, pH 7.2, and concentrated back to the original volume of serum using an Amicon centriprep 30 concentrator (Amicon, Beverly, MA).

Example 1, page 22, lines 16-34.

The claims find support in the specification which provides the first evidence that naturally occurring antibodies are targeted against the N-acetylglucosamine portion of group A

Streptococci polysaccharide. It also provides the first evidence that they can be used to produce passive immunity because they produce a bactericidal response in the presence of complement and phagocytes.

> Absorption Experiments: In an effort to determine which part of the streptococcal carbohydrate moiety was responsible for the bactericidal activity, human sera were absorbed with N-acetylglucosamine coupled sepharose beads as described in the methods section. Absorbed and non-absorbed sera were then used in the standard bactericidal assay. Figure 7 shows the results of these experiments. The unabsorbed serum clearly enhanced phagocytosis of the streptococci. In contrast, the serum absorbed with the N-acetylglucosamine coupled beads removed the opsonizing antibodies. As a viability control, normal rabbit serum did not enhance phagocytosis. These experiments indicate that the antibodies directed against the non-reducing terminal N-acetylglucosamine residue on group A carbohydrate were extremely important in the opsonophagocytosis of group A Streptococci in our bactericidal assays. To confirm these results, the antibodies from selected sera which had been absorbed to the N-acetylglucosamine affinity column were eluted and used in the bactericidal assay. As also shown in Figure 9, these experiments demonstrated that N-acetylglucosamine specific antibodies eluted from the affinity column were capable of partially restoring the opsonophagocytic bactericidal activity of the serum.
> Using methods designed to measure both precipitating and non-precipitating antibodies reactive to group A Streptococcal carbohydrate, this carbohydrate was covalently linked to phosphatidylethanolamine and incorporated into a liposome capable of binding to microtiter plates. This method clearly demonstrates that the majority of human sera contain antibodies to group A Streptococcal polysaccharide.

Example 1, page 25, line 31 to page 26, line 26.

Claims 76 through 79 relating to the use of antibodies from serum having specific titers above 40,000, 75, 000, 100,000 or 200,000 are supported by the specification, for example, in Figure 7, Figure 9 and Example 1, page 25, lines 1-6. Figure 7 shows 80% killing in the bactericidal assay by serum having titers over 200,000. Although the specification reports that

some of the serum with titers of about 40,000 were not bactericidal, the specification also states that "[o]ne serum with a CHO titer of 40,000 did promote phagocytosis but the degree of killing was far less than that observed with high titered anti-CHO sera." Page 25, lines 4-6. Figure 9 shows the opsonophagocytic index of rabbit serum. Although it is noted that there was a lack of phagocytosis with serum having titers less than 50,000, it is noted that a gradual increase in phagocytosis with serum having titers of 75,000 and complete phagocytosis with serum having titers of 100,000.

Evidence regarding the antibody titer and bactericidal activity of naturally occurring antibodies is further disclosed as follows:

> The question of whether these carbohydrate antibodies promote opsonophagocytosis of group A Streptococci has been answered affirmatively and the degree of opsonization correlated well with the level of anti-carbohydrate antibodies. ELISA titers of less than 100,000 were generally ineffective while the majority of sera with titers greater than 200,000 promoted phagocytosis. An important observation was the fact that this opsonophagocytosis was not limited to one serotype of group A Streptococci since at least three other strains of different serotypes were also phagocytized. The importance of the role of the N-acetyl-glucosamine reactive antibodies in opsonization was attested to by the fact that the absorption of these antibodies from human sera completely abolished the bactericidal activity of the sera and that, when these antibodies were eluted and added back to the bactericidal assays, killing was restored.

Page 27, line 24 to page 28, line 5.

The claims are further supported in the specification by Example 1 which describes bactericidal assays that include the presence of complement and phagocytes which participate in the opsonophagocytic response resulting in killing of the streptococcal bacteria.

Patentability of the additional claims over the prior art is based on the same grounds relied on for the presently allowed claims in the parent application. As explained by Dr. McCarty (Declaration attached as Exhibit 1), prior to applicants' invention the prior art did not consider antibodies to the carbohydrate portions of group A Streptococcal bacteria to be passively protective. Thus, the art provided no motivation to use antibodies to the carbohydrate portions of group A Streptococcal bacteria against group A Streptococcal infection.

In view of the above, applicants believe the claims stated above are supported. No new matter is added by this amendment. Entry thereof is respectfully requested.

AUTHORIZATION

No additional fees are believed necessary with this submission, however, if fees are required please charge any fees required in connection with this submission to Deposit Account No. 13-4500, Order No. 2016-4005US1.

Respectfully submitted,

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